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# The diversity of *Campylobacter* spp. throughout the poultry processing plant

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## Abstract

Campylobacteriosis is the leading food-borne disease in developed countries, and poultry are a major source for human infection. The diversity of *Campylobacter* on chicken carcasses during processing may lead to isolates that are able to survive abattoir processing. This has important implications for public health and adds a further layer to the complexity of the epidemiology of campylobacteriosis. The diversity of the *Campylobacter* spp. populations on broiler carcasses was studied at three different stages of processing (post-bleed, post-scald and post-chill) in three UK processing plants, using the pulsed-field gel electrophoresis (PFGE) KpnI enzyme. One hundred and sixty *Campylobacter* strains from 3 processing plants were identified as *C. jejuni* (92.3%) with 27 PFGE subtype profiles recovered from carcasses at the post-bleed point. Change in populations was identified when carcasses move towards the end of poultry processing. Seven *C. jejuni* genotypes were able to survive the scalding tank stage process, and 5 genotypes surviving the entire poultry process. Confirmation by PFGE gives information on the genotypic profiles of *C. jejuni* on chicken carcasses and how they change according to the temperatures exposed to during processing. Diversity within *C. jejuni* populations produces genotypes that adapt to tolerate the processing environment, and these may be capable of causing human disease. Understanding more about the genotypes that survive the processing will have important implications for public health.

## KEYWORDS

*Campylobacter*, heat tolerance, PFGE type, poultry processing, survival

## 1 | INTRODUCTION

*Campylobacter* spp. are a main cause of acute bacterial diarrhoea and are among the most common bacterial food-borne infection worldwide (WHO, 2020), and *C. jejuni* and *C. coli* are known as thermophilic which are the most frequently reported in human diseases (WHO, 2020). This bacterium is highly adapted to live at 41.5°C, the body temperature of the chicken, where they have developed the ability to survive and colonize the chicken gut, through the use of several genes and proteins

that are essential in the cellular response (Hermans, van Deun, Martel, et al., 2011). Once *Campylobacter* spp. are present in a broiler flock, the bacterium spreads rapidly and the caecum of the birds can be colonized by up to 10<sup>9</sup> CFU/g without any visible clinical symptoms (de Boer et al., 2002; Hermans, van Deun, Messens, et al., 2011). There is an increasing body of evidence which has shown extra-intestinal spread to edible tissues such as the liver (Firlieyanti et al., 2016), which poses a risk to human health. Whilst chickens have no visible clinical symptoms, they do mount both an adaptive and innate

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immune response to *Campylobacter* (Humphrey et al., 2014; Williams et al., 2013). After colonization, *Campylobacter* spp. remain in the birds throughout their life cycle until slaughter, despite interventions both on the farm and in the processing plant, such as strict biosecurity and disinfection (Newell et al., 2011). Large numbers of *Campylobacter* spp. are present throughout the processing plant making avoidance of cross-contamination between birds and flocks almost impossible (Elvers et al., 2011). This explains the high correlation between poultry products and human infections (Coward et al., 2008; Stern, 2008). This viewpoint is supported by the EFSA (2011) that found more than 80% of chicken carcasses at retail sale in the United Kingdom were contaminated by *Campylobacter* spp.

The processing plant has been recognized as a high-risk area which is involved with cross-contamination between birds. García-Sánchez et al., (2017) reported that the de-feathering and evisceration machines were very effective in spreading *Campylobacter* spp. between carcasses. Herman et al., (2003) found that the same *Campylobacter* spp. were identified both during rearing on the farm and on the final product after processing and concluded that a correlation exists between the contamination of broiler chickens during rearing and after processing. This indicates that *Campylobacter* is able to survive during processing and control measures are not always effective.

Molecular epidemiological techniques: for examples, ribotyping, PFGE, flagellin typing (fla typing) and multilocus sequence typing (MLST) (Dingle et al., 2001; Wassenaar & Newell, 2000) have shown *Campylobacter* genotypes are widely diverse, leading to an increased survival potential under environmental stressors such as processing. In one study, Parkhill et al., (2000) concluded that thermophilic *Campylobacter* spp., can survive in the environment via genetic diversity and a high rate of variation in homopolymeric tracts. Furthermore, diversity in the genotypes provides a bacterial population with genome plasticity that may increase the adaptation for survival in a hostile environment (de Boer et al., 2002). In addition, Newell et al., (2001) stated that in the UK, individual broiler chickens can be colonized by more than one subtypes of *C. jejuni* or *C. coli* but in low number of campylobacter of the same subtypes. Therefore, identifying *Campylobacter*

### Impacts

- A high level of genetic diversity was found among the *Campylobacter* isolates with 27 PFGE profiles recovered from chicken carcasses at the post-bleed point using PFGE *KpnI* enzyme.
- Seven *C. jejuni* subtypes were able to survive the scalding stage process (exposing the birds to a temperature of around 52°C, loosen the feathers).
- Changes in *Campylobacter* populations were identified when carcasses move towards the end of poultry processing with 5 *C. jejuni* subtypes surviving the entire poultry process. This emphasizes the importance of reducing *Campylobacter* population in poultry abattoirs.

spp. in poultry by comparing the genetic profiles can help to understand the transmission routes of these bacteria, allowing the development of suitable preventive methods (Johnsen et al., 2006).

Analysis of *Campylobacter* genotypic diversity from processing plants and broiler chickens showed both low diversity among *Campylobacter* isolates and only two different profiles have been identified. There is a large diversity among broiler flocks with four different profiles, when campylobacters on carcasses were analysed during process using PFGE (Normand et al., 2008). Although some subtypes were found in post-chill stages and had survived processing, it was concluded that *Campylobacter* genetic diversity declined through processing (Hunter et al., 2009). The objectives of this study were to identify if there was a change in *Campylobacter* populations on carcasses through the processing plant by examining the diversity among *Campylobacter* isolates collected at post-bleed, post-scald and post-chill stages of the processing chain at three UK commercial poultry processors to identify *Campylobacter* genotypes that are capable of surviving.

	Primers	Sequences (5'–3')
<i>C. jejuni</i> HipO (jejuni) probe	HipO F HipO R	GTT ATT GGA AGG GGT GGT CA GCC ACA ATA AGC AAA GAA GCA ROX 5AGT GCT CCA GAA AAG GCA AA 3' BHQ2
<i>C. coli</i> GlyA (coli) probe (2)	GlyA F GlyA R	GCG TGA ATT TAG CGG AAA AG TAA GGG CAG GCG TTC CTA AT FAM 5'GTG CCT GGC GAA ACT AGA AG 3' ECLIP
<i>C. upsaliensis</i> <i>C. upsali</i> (upsal) probe (2)	<i>C. upsali</i> F <i>C. upsali</i> R	CGA TGA TGT GCA AAT TGA AGC TTC TAG CCC CTT GCT TGA TG CY5 5'GAA GCT AAA ATC GGC AAT GG 3' BBQ
<i>Campy</i> 16S Lund16S pmgb probe	Lund 16S FLund 16S R	5'-GGA AGG AAG GGT TAA GTG TTA-3' 5'-GAG TTA GCC GGT GCT TCT T-3' HEX 5'-GCG AGT AAC GTC AAT GTT CAG TGC BHQ1-3'

**TABLE 1** Primers and probes for speciation of *Campylobacter* spp

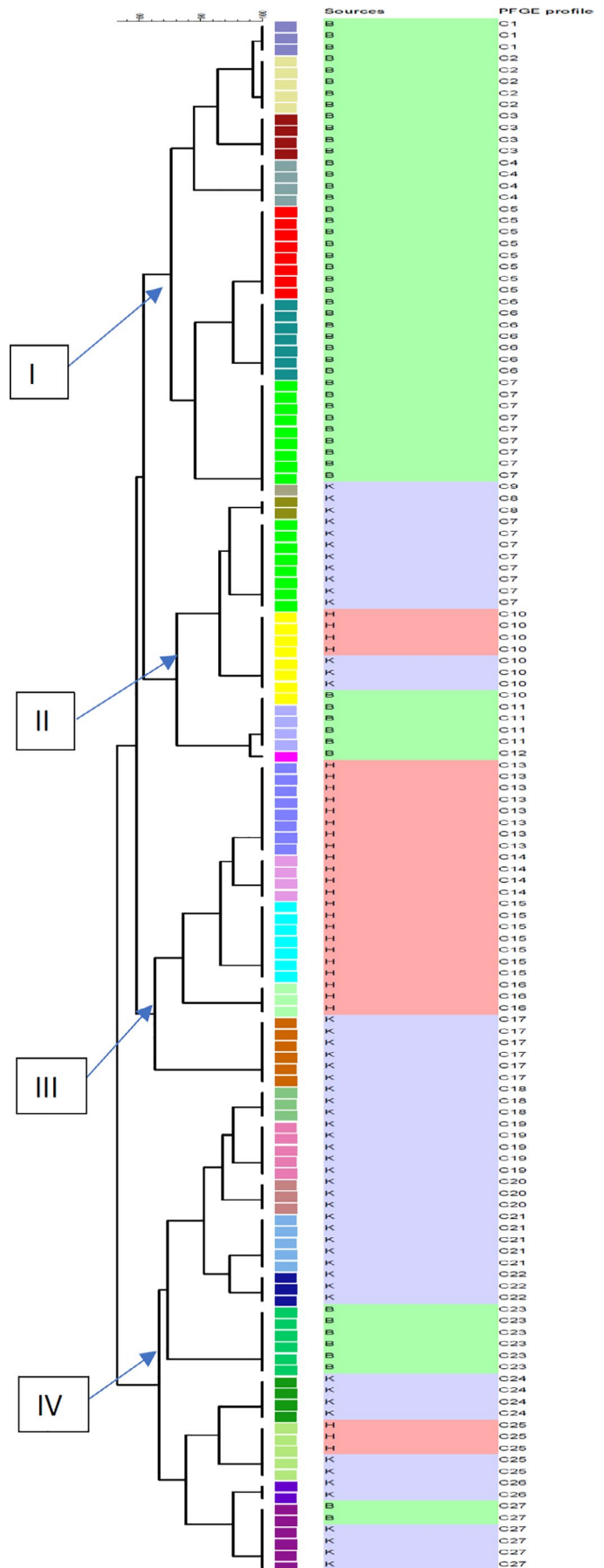
## 2 | MATERIALS AND METHODS

### 2.1 | Chicken Samples from processing plants

Five neck skin samples per flock from three different flocks (F1, F2, F3) were taken at three specific stages of processing (post-bleed, post-scald and post-chill) in three commercial processing plants (B, K, H), located in England, UK, from November 2012 to October 2013. The samples were taken from the same flock of chickens from arrival at the slaughterhouse to packing for retail sale as part of a University of Bristol study funded by the Food Standards Agency (FSA). A total of 25 g of neck skin per bird, using sterile scissors, was removed and placed into individual bags from the first flock of the day. All samples were transported to the laboratory in an insulated box containing ice packs and processed on the day of slaughter. One hundred and thirty-five neck skin were sampled, and from each, two to four colonies were taken; in total 521 colonies were recovered. For logistical reason, 160 isolates were randomly chosen to be representative of *Campylobacter* diversity across the abattoirs.

### 2.2 | Bacteria and culture conditions

As described by Elvers et al., (2011), neck skin samples were plated onto modified charcoal cefoperazone deoxycholate agar (mCCDA; Oxoid Ltd). Plates were incubated at 41.5°C for 48 hr in a micro-aerobic atmosphere generated using the CampyGen gas generating sachets (Oxoid Ltd.). Colonies of *Campylobacter* spp. were identified on the basis of colony morphology on mCCDA, on which they appeared as a greyish metallic colony with a tendency to spread, and 2–4 colonies from each sample were taken. These were streaked onto two separate Columbia blood agar plates (BA; Oxoid Ltd.) containing 5% (w/v) defibrinated horse blood. One plate was incubated at 41.5°C for 48 hr in a microaerobic atmosphere generated using CampyGen (Oxoid Ltd.), and the second plate was incubated at 41.5°C for 48 hr aerobically to rule out any possibility of the



**FIGURE 1** Dendrograms representing relatedness among PFGE profiles of *KpnI* digests of 160 *Campylobacter* isolates detected on broiler carcasses in post-bleed point process from processing plants (B), (K) and (H). The dendrogram was constructed by using Dice Coefficients matrix based on the UPGMA. The strains grouped into clusters of 80% genetic similarity indicated as I II III IV\*. Clusters were defined as isolates whose restriction digests had a similarity coefficient  $\geq 80\%$ . Each letter and number represent a different genotype. Each colour on the right-hand side of the figure represents a different processing plant, plant B is in green, K is in blue and H is in pink. The coloured blocks on the left-hand side of the figure indicate the different PFGE profiles. \*The isolates were closely related, allowing them to be grouped into clusters of at least 80% genetic similarity. The similarity value at each branching in the dendrogram represents the average similarity of profiles in the branch, and 100% means the unique profiles were identical. The software GelCompare II was used



**FIGURE 2** Dendrograms representing relatedness among PFGE profiles of *KpnI* digests of *Campylobacter jejuni* genotypes detected on broiler carcasses at the post-bleed point in the process from commercial processing plant K. Clusters were defined as isolates whose restriction digests had a similarity coefficient  $\geq 80\%$ . Isolates with similar profiles indicated close genetic relationships, allowing them to be grouped into clusters of at least 80% genetic similarity. The similarity value at each branching in the dendrogram represents the average similarity of profiles in the branch, and 100% similarity means the unique profiles were identical. The software GelCompare II was used

colonies being contaminants. Any colonies that did not grow aerobically were presumed to be *Campylobacter* spp. and were further confirmed using Gram staining and Oxidase positivity. Bacteria were stored on Cryobeads (Microbank™, Pro-Lab diagnostics) at  $-80^{\circ}\text{C}$  until required for further confirmation using PCR. When a culture was needed a single bead was removed from the Cryovial and placed onto a BA plate and incubated microaerobically as described above.

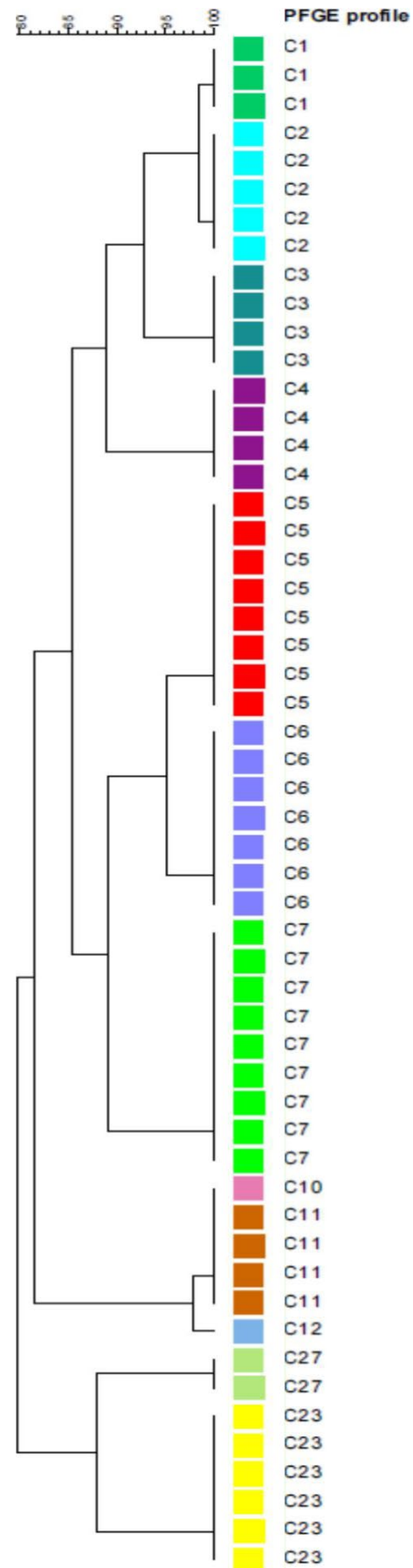
### 2.3 | Deoxyribonucleic acid (DNA) extraction

The DNA from the samples was extracted using the QIAamp® DNA extraction Kit (Qiagen). The manufacturer's protocol for purification of total DNA (Spin-Column protocol) was followed <https://www.qiagen.com/gb/resources/resourcedetail>. Eluted DNA was stored at  $-20^{\circ}\text{C}$  until use.

### 2.4 | *Campylobacter* speciation using multiplex real time PCR

A multiplex real-time PCR (RT-PCR) was used to identify the species of forty-eight isolates from plant B, which represented (2x) 24 PFGE *Campylobacter* genotypes chosen from post bleed (12 PFGE genotypes), post-scald (7 PFGE genotypes) and post-chill (5 PFGE genotypes) stage samples. Using a combination of in-house designed primers for *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter upsaliensis* and previously published primers for the 16S rRNA gene of *Campylobacter* (Lund et al., 2004). The primers and probes used in the multiplex PCR are described in Table 1.

Real-time PCR was performed with 1  $\mu\text{l}$  of DNA and 24  $\mu\text{l}$  of Master Mix (Qiagen) for each reaction as follows: 0.5  $\mu\text{l}$  of each of forward and reverse primers and 0.25  $\mu\text{l}$  probe targeting *C. jejuni*, *C. coli*, *C. upsaliensis* and *Campylobacter* 16S rDNA gene, respectively, 12.5  $\mu\text{l}$  of Brill II PCR mastermix (Agilent Technologies), 7.75  $\mu\text{l}$  nuclease-free water and 1  $\mu\text{l}$  of samples of extracted DNA were added in a final volume of 25  $\mu\text{l}$  for each reaction. The RT-PCR application was performed on Stratagene Mx3005P machine (Agilent Technologies). The quantitative PCR thermal cycle conditions were  $95^{\circ}\text{C}$  for 10 min followed by 40 cycles of  $95^{\circ}\text{C}$  for 15 s,  $58^{\circ}\text{C}$  for 30 s and  $72^{\circ}\text{C}$  for 30 s, with final cycle of 5 min at  $72^{\circ}\text{C}$ , using a



**FIGURE 4** Dendrograms representing relatedness among PFGE profiles of *KpnI* digests of *Campylobacter jejuni* genotypes detected on broiler carcasses at the post-bleed point in the process from commercial processing plant B. Clusters were defined as isolates whose restriction digests had a similarity coefficient  $\geq 80\%$ . Isolates with similar profiles indicated close genetic relationships, allowing them to be grouped into clusters of at least 80% genetic similarity. The similarity value at each branching in the dendrogram represents the average similarity of profiles in the branch, and 100% similarity means the unique profiles were identical. The software GelCompare II was used



Birds/carcass	PFGE profile on single neck skin/ four colonies*	Number of genotypes on carcass	Bird flock*
1A5	C23, C27	2	F1
1A4	C2, C23, C27	3	F1
1A2	C4	1	F1
1A1	C1, C3, C7	3	F1
1A3	C1, C12	2	F1
2A5	C6, C23	2	F2
2A3	C6, C7	2	F2
2A2	C7, C2, C6	3	F2
2A4	C2, C3, C6	3	F2
2A1	C5	1	F2
3A2	C10, C7	2	F3
3A3	C7, C11	2	F3
3A5	C7, C11	2	F3
3A1	C2, C5, C3	3	F3
3A4	C2, C5	2	F3

\* Flock 1 = F1, flock 2 = F2 and flock3 = F3.

**TABLE 3** The diversity index values of *Campylobacter jejuni* strains from post-bleed, post-scald and post-chill

Abattoir processing point	N isolates	N genotypes	Simpson's index of diversity**
Post-bleed	60	12	0.91 <sup>a</sup>
Post-scald	60	7	0.84 <sup>b</sup>
Post-chill	60	5	0.74 <sup>b</sup>

\*Values with different letters are significantly ( $p < .05$ ) different when tested using Fisher's exact test.; \*\*Simpson's index is used to illustrate genetic diversity as outlined in material and methods, where 0 = no genetic diversity; 1 = high genetic diversity.

Mx3005p system (Agilent Technologies). The data analysis was performed using the MxPro software (Agilent Technologies).

## 2.5 | *Campylobacter* diversity analysis

### 2.5.1 | Pulsed-field gel electrophoresis

All the DNA preparations, restriction endonuclease digestions and pulsed-field gel electrophoresis (PFGE) were carried out as described by Michaud et al., (2001) and PFGE Standard Operating protocol for PulseNet (2013).

**TABLE 2** Genotypes of *Campylobacter jejuni* detected on broiler neck skins from different broiler flocks in a commercial processing plant (B) at post-bleed point process in the UK, using PFGE *KpnI* digestion

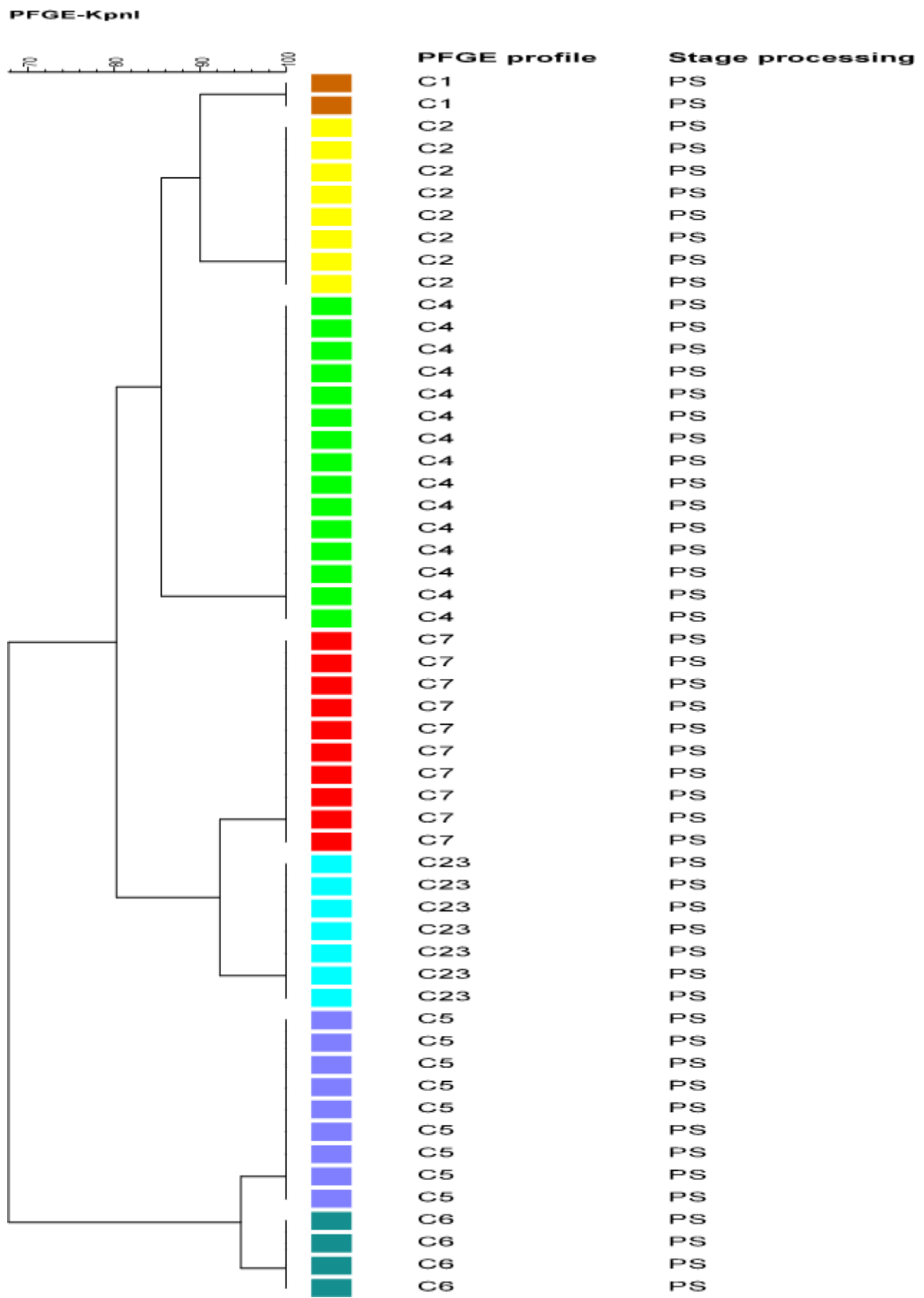
### 2.5.2 | Pulsed-field gel electrophoresis analysis

Hundred and sixty isolates were randomly chosen to be representative of *Campylobacter* diversity across three commercial processing plants (B, K, H). The *KpnI* PFGE restriction profile patterns were compared using GelCompar II gel analysis software (Applied Maths, Sint-Martens-Latem, Belgium). Based on band position, the similarity between profiles was identified and derived from the Dice correlation coefficient, with maximum tolerance of 2% which was used to compensate for between-gel variance and 5% optimization. A coefficient matrix was used to generate dendrograms based on the unweighted pair group method using arithmetic averages (UPGMA) (de Boer et al., 2000). The estimation of genetic diversity of the *Campylobacter* populations was calculated using Simpson's index as described by Hunter (1990).

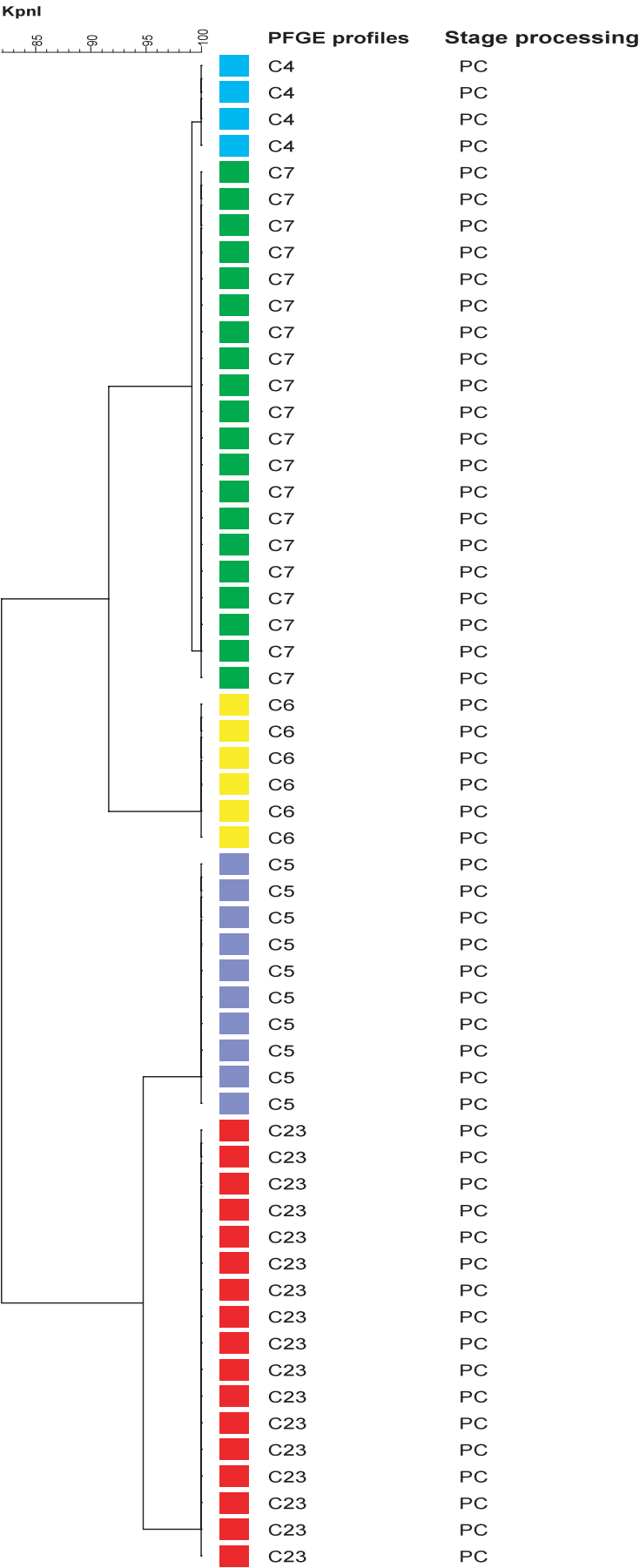
## 2.6 | Statistical analysis

Determination of  $p$ -value in different processing stages was carried out using the website <http://www.graphpad.com/quickcalcs/catMenu/>. For the diversity index values, Fisher's analysis, a 2 x 2 contingency table and one-tailed  $p$ -value were used.  $p$  values were considered to be significant below .05.

**FIGURE 5** Dendrograms representing relatedness among PFGE profiles of *KpnI* digests of *Campylobacter jejuni* genotypes detected on broiler carcasses at the post-scald point in the process from commercial processing plant B. Clusters were defined as isolates whose restriction digests had a similarity coefficient  $\geq 80\%$ . Isolates with similar profiles indicated close genetic relationships, allowing them to be grouped into clusters of at least 80% genetic similarity. The similarity value at each branching in the dendrogram represents the average similarity of profiles in the branch, and 100% similarity means the unique profiles were identical. The software GelCompare II was used

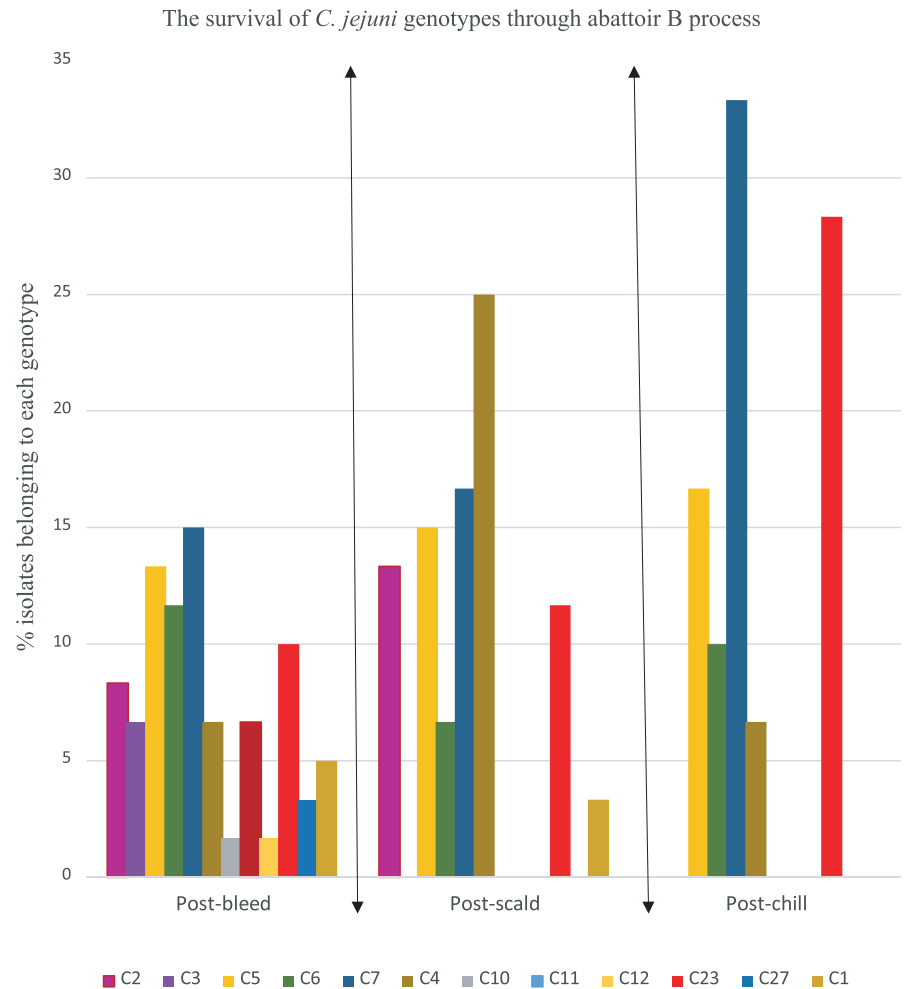






**FIGURE 6** Dendrograms representing relatedness among PFGE profiles of *KpnI* digests of *Campylobacter jejuni* genotypes detected on broiler carcasses at the post-chill point in the process from commercial processing plant B. Clusters were defined as isolates whose restriction digests had a similarity coefficient  $\geq 80\%$ . Isolates with similar profiles indicated close genetic relationships, allowing them to be grouped into clusters of at least 80% genetic similarity. The similarity value at each branching in the dendrogram represents the average similarity of profiles in the branch, and 100% similarity means the unique profiles were identical. The software GelCompare II was used

**FIGURE 7** The survival of *Campylobacter jejuni* subtypes throughout plant B at the three different processing stages. Each coloured bar is a genotype as indicated by the key at the bottom of the graph. The left side of vertical axis represents the percentages of the *C. jejuni* populations assigned to specific genotype at specific stage of processing. These populations of each *Campylobacter* genotype change during processing stages, and the surviving genotype makes up more of the populations



### 3 | RESULTS

*Campylobacter* was detected in 490/521 (94%) of the neck skins analysed, irrespective of processing plant. From these isolates, a subset was analysed from all processing plants B ( $n = 60$ ), H ( $n = 45$ ) and K ( $n = 55$ ), using multiplex RT-PCR and 160 of these isolates were identified as *C. jejuni* and further characterized using PFGE.

#### 3.1 | Genotype diversity from single birds

One hundred and sixty *C. jejuni* isolates were examined that belonged to three processing plants B ( $n = 60$ ), H ( $n = 45$ ) and K ( $n = 55$ ) located in England, and the diversity of *C. jejuni* populations on carcasses flocks in the same abattoir and between abattoirs revealed that 15% of carcasses had only one *C. jejuni* PFGE genotype present. With a maximum of four different colonies per carcass tested, 52.5% carried two different *C. jejuni* PFGE genotypes and 32.5% carried three different *C. jejuni* PFGE genotypes. PFGE typing recognized a total of 27 distinct profiles among 160 *C. jejuni* isolates from three chicken abattoirs (Figure 1). Each individual genotype was identified with (C) letter and number (1–27). PFGE dendrograms demonstrated the relationship between these isolates

and PFGE diversity showed a clustering of four *C. jejuni* genotypes (Figure 1): 80% similarity was observed between the clusters.

PFGE profiles between the slaughterhouses (B, H and K) for each bird carcass examined showed a considerable diversity among *C. jejuni* isolates with one carcass having three genotypes present (Figure 1). However, there were also some carcasses carrying only one genotype and the profiles C7 and C23 were more common. The PFGE of *C. jejuni* genotypes were more diverse at the first point of processing. Figures 2, 3 and 4 show a relationship between the isolates at each processing plant.

#### 3.2 | Diversity within the poultry processing plant

Plant B was selected for further study as it had the largest genetic diversity at all three process stages and gave the opportunity to ascertain diversity of *Campylobacter* genotypes. *Campylobacter jejuni* diversity was determined from three flocks at post-bleed, post-scald and post-chill from the processing line of plant B. A total of 12 PFGE genotypes were found in post-bleed samples, seven PFGE genotypes were found post-scald and five PFGE genotypes were found post-chill (Table 2). The isolates were shown to be highly genetically diverse from the birds at post-bleed (Figure 4), but there was less genetic

diversity among *C. jejuni* strains at post-chill (Figure 6). Table 3 represents the number of *C. jejuni* isolates from plant B and the number of *C. jejuni* genotypes detected from post-bleed, post-scald and post-chill of the chicken processing line, respectively. Figures 4, 5 and 6 show a relationship between these isolates at each sample point.

The diversity index value of *C. jejuni* strains from post-bleed was 0.908, and this index was lower at the post-scald and post-chill point in the processing line (0.837 and 0.746, respectively; Table 3). This difference was still evident as most genotypes were found commonly on carcasses in early processing rather than later in the post-chill stage.

Thus, *C. jejuni* subtypes obtained from post-bleed were statistically more highly diverse than those sampled from post-scald and post-chill (0.016 and 0.002, respectively) when using the Fisher's exact test.

### 3.3 | Identification of *Campylobacter jejuni* PFGE genotypes surviving processing

The survival of *C. jejuni* PFGE subtypes from carcasses at post-bleed, post-scald and post-chill at plant B is shown in Figure 7. The proportion of the total *C. jejuni* population is considerably lower in the post-scald and post-chill stage compared with the post-bleed stage (Figure 7). The highest diversity of *C. jejuni* subtypes was found on carcasses early in the processing chain, although some *C. jejuni* subtypes at post-bleed were not able to survive later (post-chill). The *C. jejuni* C3, *C. jejuni* C10, *C. jejuni* C11, *C. jejuni* C12 and *C. jejuni* C27 sub-types were not found in post-scald, whilst *C. jejuni* C1 and *C. jejuni* C2 were not seen at the post-chill stage. Only *C. jejuni* C4, *C. jejuni* C5, *C. jejuni* C6, *C. jejuni* C7 and *C. jejuni* C23 sub-type seemed to have survived the entire process and were detected at all sampling points.

The genotypes remaining at post-chill were in the following percentages *C. jejuni* C4 6.7%, *C. jejuni* C5 16.7%, *C. jejuni* C6 10%, *C. jejuni* C7 33.3%, and *C. jejuni* C23 28.3% and made up more of the population at this stage. *C. jejuni* C4 from plant B made up the largest percentages of the *C. jejuni* subtype samples post-scald (25%). The dominant genotypes at post-chill were *C. jejuni* C7 33.3% and *C. jejuni* C23 28.3% (Figure 7).

## 4 | DISCUSSION

*Campylobacter* has a long history of being associated with chickens, with the number of human cases continuing to rise despite interventions (WHO, 2018). There is a need to understand more about the relationship between *Campylobacter* and chickens and how the bacterium survives through poultry processing. The PFGE micro-restriction profiles revealed a considerable diversity among *Campylobacter* isolates obtained earlier in the processing chain. The total number of different PFGE profiles recovered from carcasses at the post-bleed point in the three processing plants was high ( $n = 27$ ), with two to four different types being isolated from

a single carcass. This indicates that there are significant genetically diverse populations of *Campylobacter* on carcasses from poultry processing plants, including those from the same flock (Table 2). These results are in agreement with Marotta et al., (2014) who found a high level of genotypic diversity of *Campylobacter* populations present in chicken carcasses throughout poultry production. The results demonstrated that individual carcasses could harbour multiple strains of *Campylobacter* spp. at the post-bleed point.

*Campylobacter* isolates from abattoirs H, B and K were highly diverse at the beginning of the slaughter process, probably because samples originally came from different broiler farms and there was carry over of the *Campylobacter* populations between the rearing cycles and/or flocks (Marotta et al., 2014). The possibility exists that cross-contamination could also occur from the transport crates or the auto-killer, as they have been shown previously to be contaminated (Mead et al., 1994; Stern et al., 1995). However, another study suggested that cross-contamination during poultry processing was undetectable on the end product (Evers et al., 2011). The present study showed that the isolates from abattoirs fell into four major PFGE groups clustering at 80% similarity, thus indicating all isolates were related irrespective of the abattoir or the original farm (Figure 1; Denis et al., 2008).

A review of the literature on the effects of different processing steps in *Campylobacter* spp. diversity on chicken carcasses at abattoirs revealed that the scalding and chilling stages consistently decreased the diversity of *Campylobacter* (Chan et al., 2001; Guerin et al., 2010; Hunter et al., 2009). The present study has shown that the diversity of *Campylobacter* PFGE genotype populations on chicken carcasses through the plant slaughtering line changed as the carcasses moved through the system (Figure 7) matching the results of the previous studies of Hunters and workers. The results of this study suggest that the process stages could have influenced the genotype diversity found on carcasses, indicating that the process stage may have an impact on survival of particular strains. This reduction in *Campylobacter* genotypes is most likely due to the inability of the majority of isolates to survive the heat and cold stress that they are subjected to during processing. Seven *C. jejuni* PFGE genotypes were identified in this study at the scalding stage (Figure 5), suggesting that those that survived this part of the process are the *Campylobacter* genotypes that are more heat tolerant and so are therefore more likely to be recovered from the end product.

Further changes in levels of *C. jejuni* population diversity on carcasses after the scalding tank occurred when carcasses moved through the system into the chilling stage. Results showed a change in *C. jejuni* genotype populations; in particular, three PFGE genotypes *C. jejuni* C5, *C. jejuni* C7 and *C. jejuni* C23 were more dominant at this stage (Figure 7). Cold stress may act as a selective force which permits cold-tolerant *Campylobacter* to survive (Chan et al., 2001). This difference was still evident as most genotypes were found commonly on carcasses in early processing rather than later in the post-chill stage. These findings are supported by the study of Hunter et al., (2009) who demonstrated that chilling can be a factor in the reduction of *Campylobacter* diversity.

Interestingly, *C. jejuni* genotypes *C. jejuni* C4, *C. jejuni* C5, *C. jejuni* C6, *C. jejuni* C7 and *C. jejuni* C23 were found in both scalding and chill stages in abattoir B (Figure 7). Thus, these five genotypes are able to survive the higher temperature of 55°C and low temperature 6°C which they are exposed to during processing. This result indicates that survival at low and high temperatures could be related as previously reported by Zhang and Rock (2008) and Hughes et al., (2010) and that the bacterium can survive in a wide range of physical environments through the controlled biophysical properties of their membrane phospholipids. Some isolates could be detected throughout poultry processing and some were only detected up to the scalding stage and were not recovered post bleed. While the levels fluctuated these results show that only the more resilient isolates can survive (Figure 7). This is in agreement with Newell et al., (2001), who found that the resistance of some *Campylobacter* subtypes during poultry processing varied between strains and the more robust strains survived through carcass chilling. Taken together, these results suggest that elimination of *Campylobacter* genotype populations on carcasses at the final stage may be related to the number of subtypes that are better able to adapt to the stress of poultry processing. It suggests that the carcasses containing more than one genotype may have had an impact on the diversity of the organism to produce strains that survive throughout the process. This could lead to identifying particular genetic elements related to the ability to survive heat and cold stress within the abattoir environment. The findings also suggest that *C. jejuni* genotypes are able to survive under these conditions and are may be capable of causing human disease.

## 5 | CONCLUSION

Different *C. jejuni* genotypes can survive at various stages of the process, and this may help to identify the factors responsible for survival at each stage. This knowledge may help to identify methods for reducing *Campylobacter* on chicken carcasses and improve processing plant hygiene and public health.

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## AUTHOR CONTRIBUTIONS

H. M. designed and carried out the experiment and wrote the manuscript. L. K. W. and E. V. K. helped in analysis and interpretation of the data and provided critical feedback and helped shape the research.

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